FORM PTO-1390
(REV. 11-94)

U S DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

ATTORNEY'S DOCKET NUMBER
8484-077-999

09/463474

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE98/02102	22 July 1998	23 July 1997

TITLE OF INVENTION
CONJUGATE FOR DIFFERENTIATING BETWEEN HEALTHY AND UNHEALTHY TISSUE

APPLICANT(S) FOR DO/EO/US
Sinn et al.

Applicant herewith submits to the United States Designated/ Elected Office (DO/EO/US) the following items under 35 U.S.C. 371:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the international Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureaus.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 37(c)(3)).

9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) (unexecuted).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☒ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information:

Verified Statement Claiming Small Entity Status (unexecuted);
Claims as Amended (1-11) under Article 34 and English translation thereof;
PCT Chapter II Request;
Request for International Preliminary Examination;
International Preliminary Examination Report;
Rule 66 Report;
PCT Demand;
International Search Report

INTERNATIONAL APPLICATION NO PCT/DE98/02102	INTERNATIONAL FILING DATE 22 July 1998	09/463474

17. ☒ The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees as follows:

09/463474 PCT/PTO 21 JAN 2000

CLAIMS				
(1)FOR	(2)NUMBER FILED	(3)NUMBER EXTRA	(4)RATE	(5)CALCULATIONS
TOTAL CLAIMS	16 - 20	0	X $ 18.00	$ 0.00
INDEPENDENT CLAIMS	4 - 3	1	X $ 78.00	78.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $ 260.00	☐

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): **CHECK ONE BOX ONLY** ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... $ 670	
☐ No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ..... $ 760	
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $970	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2) to (4) ..... $ 96	
☒ Filing with EPO or JPO search report ..... $ 840	$ 840.00
Surcharge of $130.00 for furnishing the National fee or oath or declaration later than 20 30 mos. from the earliest claimed priority date (37 CFR 1.492(e)).	
TOTAL OF ABOVE CALCULATIONS	= 918.00
Reduction by 1/2 for filing by small entity, if applicable. Affidavit must be filed also. (Note 37 CFR 1.9, 1.27, 1.28).	- $
SUBTOTAL	= 918.00
Processing fee of $130.00 for furnishing the English Translation later than 20 30 mos. from the earliest claimed priority date (37 CFR 1.492(f)).	+
TOTAL FEES ENCLOSED	$ 918.00

☐ a. ☐ A check in the amount of $_ to cover the above fees is enclosed.

b. ☒ Please charge Deposit Account No. 16-1150 in the amount of $ 918.00 to cover the above fees. A copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 16-1150. A copy of this sheet is enclosed.

18. ☐ Other instructions

n/a

19. ☒ All correspondence for this application should be mailed to

PENNIE & EDMONDS LLP
1155 AVENUE OF THE AMERICAS
NEW YORK, NEW YORK 10036-2711

20. ☒ All telephone inquiries should be made to (212) 790-2803

Birgit Millauer NAME Laura A. Coruzzi For: Laura A. Coruzzi (Reg. No. 30,742)	SIGNATURE	43,341 REGISTRATION NUMBER	January 21, 2000 DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: ☒ Application of: Sinn *et al.*
☐ Patent of:

☐ Application No.: 09/463474 Group Art Unit: To be assigned
☐ Patent No.:

☒ Filed: January 21, 2000 Examiner: Herewith
☐ Issued:

For: CONJUGATE FOR DIFFERENTIATING BETWEEN HEALTHY AND UNHEALTHY TISSUE

Attorney Docket No.: 8484-077-999

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
[37 CFR 1.9(f) and 1.27(d)] - Nonprofit Organization

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

Name of organization Deutsches Krebsforschungszentrum Stiftung des öffentlichen Rechts
Address of organization Im Neuenheimer Feld 280, D-69120 Heidelberg Germany

Type of organization
☐ University or other institution of higher education
☐ Tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3))
☐ Nonprofit scientific or educational under statute of state of the United States of America
(Name of state _____)
(Citation of statute _____)
☒ Would qualify as tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3)) if located in the United States of America.
☐ Would qualify as nonprofit scientific or educational under statute of state of the United States of America if located in the United States of America
(Name of state _____)
(Citation of statute _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled CONJUGATE FOR DIFFERENTIATING BETWEEN HEALTHY AND UNHEALTHY TISSUE by inventor(s) Hannsjörg Sinn, Hans-Hermann Schrenk, Andreas Wunder, and Gerd Stehle described in

☐the specification filed herewith
☒ application no. 09/463474 filed January 21, 2000.
☐ patent no. issued

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization identified above and/or there is an obligation under contract or law by the inventor(s) to convey rights to the nonprofit organization identified above with regard to the invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

FULL NAME_____
ADDRESS_____
_____

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME_____
ADDRESS_____
_____

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28 (b)]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, and patent issuing thereon, or any patent to which this verified statement is directed.

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1155 Avenue of the Americas
New York, N.Y. 10036-2711

Direct Telephone calls to:
(212) 790-9090

Name of person signing: Prof.Dr.med.Harald zur Hausen | Dr. rer.pol. Josef Puchta
Title of person other than owner: Chairman a.Scient. Member | Adm. Member of the Board
Address of person signing: Eichenstr. 1, 69 483 Waldmichelbach | Eichenweg 12a, 69198 Schriesheim
Signature_____ Date: May 08, 2000

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

Express Mail No.:EL 236 252 960 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Sinn *et al.*

Serial No.: UNASSIGNED

Group Art Unit: UNASSIGNED

Filed: HEREWITH

Examiner: UNASSIGNED

For: CONJUGATE FOR DIFFERENTIATING BETWEEN HEALTHY AND UNHEALTHY TISSUE

Attorney Docket No.: 8484-077-999

PRELIMINARY AMENDMENT UNDER 37 C.F.R. §1.111

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In accordance with Rule 111 of the Rules of Practice, please consider and enter the following amendments and remarks.

AMENDMENTS

IN THE CLAIMS:

Please cancel claim 11.

Please amend the claims as follows:

1. (Amended) A conjugate [,] for distinguishing unhealthy tissue from healthy tissue comprising a fluorescent compound, a connector and a carrier, wherein the fluorescent compound and the carrier are [connected] joined via [an acidic ester or acidic amide bond or an enane bridge, the carrier is selected from the group consisting of serum albumin or a

polyether, and the compound in the conjugate has an excitation wavelength of 630 nm or more and/or 450 nm or less] the connector, and the connector comprises an acidic ester, an acidic amine bond or an enane bridge.

2. (Amended) The conjugate [according to] of claim [1] 13, [characterized in that] wherein the serum albumin [is] comprises a human serum albumin.

3. (Amended) The conjugate [according to] of claim [1] 14, [characterized in that] wherein the polyether [is] comprises a polyethylene glycol.

4. (Amended) The conjugate [according to any one of claims 1 to 3, characterized in that several carriers are present] of Claim 1 wherein the conjugate comprises a plurality of carriers.

5. (Amended) The conjugate [according to any one of claims 1 to 4, characterized in that] of claim 1, wherein the fluorescent compound comprises an acid group, a hydroxyl group, an amino group or an aldehyde group.

6. (Amended) The conjugate [according to any one of claims 1 to 5, characterized in that] of claim 15, wherein the excitation wavelength is 630 to 850 nm.

7. (Amended) The conjugate [according to any one of claims 1 to 6, characterized in that] of claim 15, wherein the excitation wavelength is 320 to 450 nm.

8. (Amended) The conjugate [according to any one of claims 1 to 7, characterized in that] of claim 1, wherein the fluorescent compound [is derived from] comprises a porphyrin, a porphyrin derivative, a chlorin, a chlorin derivative, a bacteriochlorin, a bacteriochlorin derivative, a chlorophyll, a chlorophyll derivative, a phthalocyanine, a pthalocyanine derivative, a carboxy cinnamic acid, a carboxy cinnamic acid, a carboxyfluorescein, a carboxyfluorescein derivative, an acridic acid, an acridic acid derivative, a coumaric acid, a coumaric acid derivative [or] an indocyanine green or an indocyanine green derivative [as well as the derivatives thereof].

9. (Amended) The conjugate [according to any one of claims 1 to 8, characterized in that several fluorescent compounds are present] of claim 1, wherein the conjugate comprises a plurality of fluorescent compounds.

10. (Amended) A method of producing [a] the conjugate [according to any one of claims 1 to 9, characterized in that] of claim 1, wherein the fluorescent compound and the carrier are covalently bonded thereby forming [an acidic ester or acidic amide bond] the connector.

Please add new the following new claims:

--12. (New) The conjugate of claim 1, wherein the carrier comprises a protein.

13. (New) The conjugate of claim 12, wherein the protein comprises a serum albumin.

14. (New) The conjugate of claim 1 wherein the carrier comprises a polyether.

15. (New) The conjugate of claim 1, wherein the fluorescent compound has an excitation wavelength of 630 nm or greater or 450 nm or less.

16. (New) A pharmaceutical composition comprising the conjugate of claim 1 and an acceptable carrier or excipient.

17. (New) A method of distinguishing unhealthy tissue from healthy tissue, comprising administering the pharmaceutical composition of claim 16 to a subject in need thereof.--

REMARKS

Applicants respectfully request that the above-made amendments be made of record in the file history of the instant application. The amendments do not add new matter and are fully supported by the specification and the claims as originally filed.

Respectfully submitted,

Date January 21, 2000

43,341
Birgit Millauer (Reg. No.)

for: Laura A. Coruzzi (Reg No. 30,742)
PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

Enclosure

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: ☒ Application of: Sinn *et al.*
☐ Patent of:

☐ Application No.: To be assigned
☐ Patent No.:

Group Art Unit: To be assigned

☒ Filed: Herewith
☐ Issued:

Examiner: Herewith

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(Name of state _____)
(Citation of statute _____)
☒ Would qualify as tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c)(3)) if located in the United States of America.
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☒ the specification filed herewith
☐ application no. filed
☐ patent no. issued

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization identified above and/or there is an obligation under contract or law by the inventor(s) to convey rights to the nonprofit organization identified above with regard to the invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

FULL NAME_____
ADDRESS_____
_____

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME_____
ADDRESS_____
_____

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28 (b)]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, and patent issuing thereon, or any patent to which this verified statement is directed.

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New York, N.Y. 10036-2711

Direct Telephone calls to:
(212) 790-9090

Name of person signing_____
Title of person other than owner_____
Address of person signing_____
_____
Signature_____ Date_____

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

K 2575

Conjugate for Differentiating Between Healthy and Unhealthy Tissue

The present invention relates to conjugates for differentiating between healthy and unhealthy tissue, methods of producing such conjugates as well as their use.

For the treatment of unhealthy tissue, e.g. of tumors, the removal thereof is often an essential measure. For this purpose, it is necessary for the operating surgeon to recognize accurately where unhealthy tissue ends and where healthy tissue starts. However, this is often impossible. As a result, offshoots of the unhealthy tissue are overlooked, which are then the basis for another formation of the unhealthy tissue.

Therefore, it is the object of the present invention to provide a product by means of which a differentiation can be made between unhealthy and healthy tissue.

According to the invention this is achieved by the subject matters defined in the claims.

Thus, the subject matter of the present invention relates to a conjugate, comprising a fluorescent compound and a carrier, wherein the compound and the carrier are connected via an acidic ester or acidic amide bond or enane bridge (schiff base) and the compound has an excitation wavelength of 630 nm or more and/or 450 nm or less.

The expression "carrier" comprises compounds of any kind which are suited for the enrichment of the conjugate in a

certain tissue, e.g. a tumor, a focus of inflammation or in superficial, relatively small vessels, such as neovascularizations in the area of the cornea. Examples of such carriers are proteins and polyether. For forming the acidic ester or acidic amide bond with the fluorescent compound, the carrier may include hydroxyl or amino groups.

The proteins are preferably not considered foreign to the body. They may be present in native form. In the native form, the proteins have no intermolecular and/or intramolecular cross-linking. The proteins favorably have a molecular weight of up to 100,000 Dalton, particularly 30,000 to 100,000 Dalton. Furthermore, it is favorable for the proteins to be human proteins. Examples of the proteins are albumin, fibrinogen, transferrin, immunoglobulins and lipoproteins, human serum albumin (HSA) being preferred. It is also possible to use fragments of the above proteins. In addition, the sequence of the proteins and the fragments thereof, respectively, may comprise modifications of one or several amino acids over known sequences of the proteins and fragments thereof, respectively.

Examples of the polyethers are polyethylene glycols, particularly those having a molecular weight of 100 to 20,000 Dalton. The polyethylene glycols are preferably esterified or etherified with a C_1-C_{12} alkyl group, particularly with a methyl group, on the terminal hydroxyl group.

A conjugate according to the invention may have one or several, particularly 2 to 4, of the above carriers. If several carriers are present, they may be equal or differ from one another. If several polyethers are present, they will favorably be selected such that the molecular weight of all polyethers is about 20,000 Dalton or more.

The expression "fluorescent compound" comprises compounds of any kind which can be induced to display fluorescence. These compounds can also be photoactive. The compound is connected with the carrier via an acidic ester or acidic amide bond or enane bridge. For the formation thereof, the fluorescent compound may comprise an acid group, e.g. a carboxylic, sulfonic, phosphonic or arsonic acid group, a hydroxyl group, an amino group or an aldehyde group. Several of these groups may be present, which may be equal or differ from one another. The fluorescent compound is excited at a wavelength of 630 nm or more, preferably 630 to 850 nm, and particularly preferably 650 to 850 nm, and/or at a wavelength of 450 nm or less, preferably 320 to 450 nm. These wavelengths refer to the excitation wavelengths which the fluorescent compound has in the conjugate according to the invention; in a free form, their excitation wavelength may differ therefrom. Representatives of these compounds are porphyrins such as tetrasulfophenyl porphyrin (TSPP; excitation wavelength 650 nm when bound to HSA), chlorins, bacteriochlorins, chlorophylls, phthalocyanines, wherein these compounds may include metal ions as central atom. Furthermore, representatives of the fluorescent compound are carboxy cinnamic acid, carboxy fluorescein, acridine carboxylic acid, such as acridine-9-carboxylic acid, coumaric acid, such as coumarin 343, coumarin-3-carboxylic acid, and hydroxy coumarin acetic acid (excitation wavelength 365 nm when bound to HSA), and indocyanine green (excitation wavelength 805 nm when bound to HSA) as well as derivatives of the above compounds.

One or several fluorescent compounds can be present in the conjugate according to the invention. If several are present, they may be the same or differ from one another.

Particularly preferred conjugates according to the invention are shown in figures 1 to 3.

Conjugates according to the invention can be produced by covalently bonding the fluorescent compound with the carrier thereby forming an acidic ester or acidic amide bond. A person skilled in the art is familiar with methods suitable for this purpose as well as necessary materials.

If the fluorescent compound includes an acid group, the conjugates can be produced by reacting this compound with carbodiimide and hydroxy succinimide into reactive succinimidyl esters and the latter can then be converted with the carrier. In the case of conjugates having several fluorescent compounds, the succinimidyl esters can be produced jointly or separately.

The fluorescent compound is reacted with carbodiimide and hydroxy succinimide in a polar aprotic solvent, preferably dimethyl formamide or dimethyl sulfoxide (DMSO). The molar ratio of fluorescent compound : carbodiimide : hydroxy succinimide is about 1 : 1.5-3 : 5-10. The resulting succinimidyl ester is then reacted in an aqueous buffer solution, preferably $NaHCO_3$, with the carrier, such as albumin. The carrier concentration is about 10 to 70 mg/ml. The thus activated acid group can then react with OH and NH groups of the carrier thereby forming acidic amide or acidic ester bonds, conjugates according to the invention being obtained. The conjugates can be purified several times, e.g. by ultrafiltration, and finally be sterile filtered. Thereafter, they are ready for application.

Conjugates according to the invention distinguish themselves by a prolonged half life in the organism. In addition, conjugates according to the invention

accumulate in unhealthy tissue, particularly in tumoral tissue, in foci of inflammation and in superficial relatively small vessels, e.g. of neovascularizations in the area of the cornea. The fluorescent compound is exited or activated by light, so that unhealthy tissue can be made visible, whereas healthy tissue in which the conjugates according to the invention do not accumulate is not made visible. Furthermore, there is no disturbance caused by the inherent fluorescence of blood or tissue, e.g. the liver, so that the optical impression is not falsified. In addition, conjugates according to the invention, in which the fluorescent compound can be excited at 630 nm or more, have a great penetration depth.

Brief description of the drawings:

Figure 1: shows the production of a conjugate from acridine-9-carboxylic acid and human serum albumin,

figure 2: shows the production of a conjugate from coumarin 343 and human serum albumin, and

figure 3: shows the production of a conjugate from tetrasulfophenylporphin and human serum albumin.

The following examples explain the invention.

Example 1 Production of a conjugate according to the invention from acridine-9-carboxylic acid and human serum albumin

The structure and the production of the conjugate are shown in figure 1.

20 mg of acridine-9-carboxylic acid hydrate (A9CA) were dissolved in 2 ml DMSO and about 100 mg of N-hydroxysuccinimide (HSI) in a molar ratio of about 10/1 as well as 30 mg N,N'-dicyclohexyl carbodiimide (DCC) in a molar ratio or about 1.5/1 were added. After about 6 hours, the formation of the hydroxysuccinimidyl ester is concluded. Following the separation of the dicyclohexyl urea (DCHU) through a solvent-resistant filter (0.2 µm), the ester is slowly added to a solution of 2 g of human serum albumin (HSA) which is dissolved in 10 ml of original solution, 10 ml of 0.34 M $NaHCO_3$ and 10 ml of methoxypolyethylene glycol (MPEG). The slight clouding resulting upon the addition disappears again after a short time. A slightly yellowish solution of a conjugate from A9CA and HSA results. The accompanying substances undesired in the finished preparation, such as excess DCC, HSI, unbound A9CA, DMSO and MPEG, are separated by means of ultrafiltration (exclusion limit 10 kD) comprising at least 4 wash steps.

Example 2: Production of a conjugate according to the invention from coumarin 343 and human serum albumin

The structure and the production of the conjugate are shown in figure 2.

20 mg of coumarin 343 (C343 = 10-carboxy-2,3,6,7-tetrahydro-1H,5H,11H-[1]benzopyranone[6,7,8,ij]-quinolizine-11-one) were dissolved in 2 ml DMSO. For this purpose, about 100 mg HSI in a molar ratio of 10/1 and 30 mg DCC in a molar ratio of about 1.5/1 were added. The ester was isolated as described in Example 1 and reacted with HSA, an intensely yellow solution of a conjugate from C343 and HSA being obtained. Undesired accompanying substances are separated as described in Example 1.

Example 3: Production of a conjugate according to the invention from tetra-(4-sulfophenyl)porphin and human serum albumin

The structure of the conjugate and its production are shown in figure 3.

Tetra-(4-sulfophenyl)porphin (TSPP) was dissolved in a concentration of 10 mg/ml in DMSO. Three times the molar amount of DCC and five times the molar amount of HSI were added to the clear dark green solution. After a reaction period of about 3 to 4 hours, the conversion into TSPP succinimidyl ester (TSPP-SE) is concluded, the resulting di-cyclohexyl urea being separated in the form of fine grains. The analytical control is carried out by means of thin-layer chromatography.

Human serum albumin (HSA, 4 g, i.e. 2 ampoules of 2 g in 10 ml each) were diluted with 2 x 10 ml of 0.17 M $NaHCO_3$ and 20 ml of methoxypolyethylene $glycol_{350}$ and charged to a 100 ml Erlenmeyer flask. The above TSPP-SE solution in DMSO was slowly added to this HSA solution with constant stirring, the initially clear solution becoming cloudy because of non-reacted DCC which is insoluble in aqueous solution. Having concluded the addition of TSPP-SE, the reaction mixture was stirred at room temperature for 30 minutes so as to complete the reaction. Thereafter, the turbid matter was separated via a sterile filter unit (Millipore, Stericup - GV, 0.22 µm Low Binding Duropore Membrane) and the low-molecular water-soluble components (DMSO, HSI and unbound TSPP) were separated by ultrafiltration via a membrane having 30 kD exclusion limit (Amicon YM 30). A conjugate according to the invention was obtained from TSPP and HSA. The linkage yield of TSPP to HSA was 85 to 90 %.

The analytical purity was controlled by means of HPLC under the following conditions:

Precolumn:	Zorbax Diol (50 x 4 mm)
Column 1:	Zorbax GF 450
Column 2:	Zorbax GF 450
Running agent:	0.2 M Na citrate, pH 7.5
Flow:	1 ml/min
Detector 1:	280 nm (for the protein)
Detector 2:	420 nm (for TSPP)

Amended Claims

1. A conjugate, comprising a fluorescent compound and a carrier, wherein the compound and the carrier are connected via an acidic ester or acidic amide bond or an enane bridge, the carrier is selected from the group consisting of serum albumin or a polyether, and the compound in the conjugate has an excitation wavelength of 630 nm or more and/or 450 nm or less.

2. The conjugate according to claim 1, characterized in that the serum albumin is human serum albumin.

3. The conjugate according to claim 1, characterized in that the polyether is a polyethylene glycol.

4. The conjugate according to any one of claims 1 to 3, characterized in that several carriers are present.

5. The conjugate according to any one of claims 1 to 4, characterized in that the fluorescent compound comprises an acid group, hydroxyl group, amino group or aldehyde group.

6. The conjugate according to any one of claims 1 to 5, characterized in that the excitation wavelength is 630 to 850 nm.

7. The conjugate according to any one of claims 1 to 6, characterized in that the excitation wavelength is 320 to 450 nm.

8. The conjugate according to any one of claims 1 to 7, characterized in that the fluorescent compound is derived from porphyrin, chlorin, bacteriochlorin,

chlorophyll, phthalocyanine, carboxy cinnamic acid, carboxyfluorescein, acridic acid, coumaric acid or indocyanine green as well as the derivatives thereof.

9. The conjugate according to any one of claims 1 to 8, characterized in that several fluorescent compounds are present.

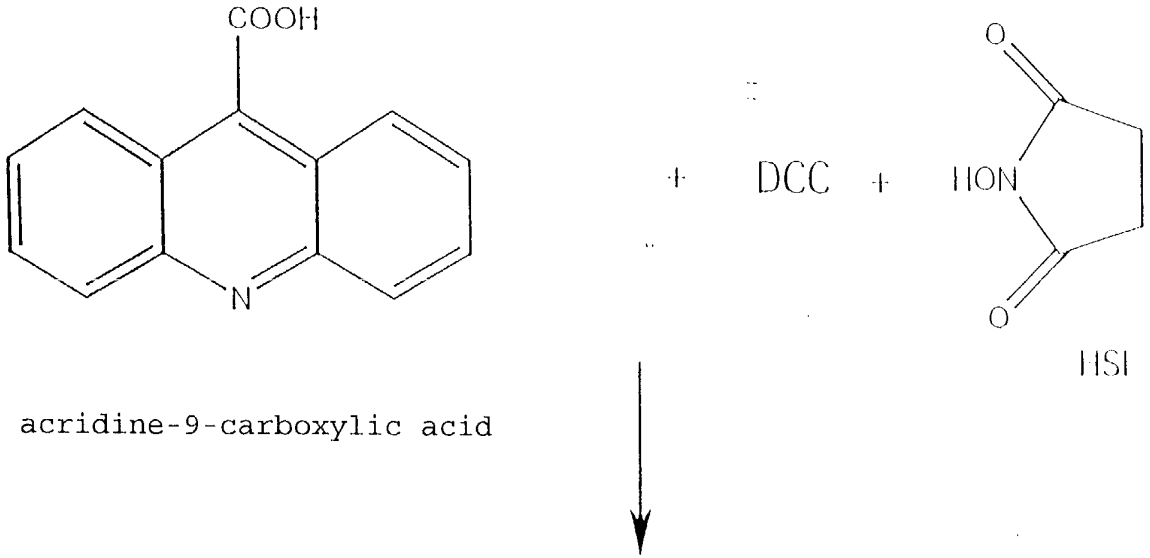
10. A method of producing a conjugate according to any one of claims 1 to 9, characterized in that the fluorescent compound and the carrier are covalently bonded thereby forming an acidic ester or acidic amide bond.

11. Use of a conjugate according to any one of claims 1 to 9 for differentiating between healthy and unhealthy tissue.

Abstract of the Disclosure

The invention relates to conjugates, comprising a fluorescent compound and a carrier, wherein the compound and the carrier are connected via an acidic ester or an acidic amide bond and the compound has an excitation wavelength of 630 nm or more and/or 450 nm or less. The invention also relates to the production of said conjugates and to the use thereof.

Figure 1:



acridine-9-carboxylic acid

Figure 1 (cont'd)

acridine-9-carboxylic acid-HSA

Figure 2:

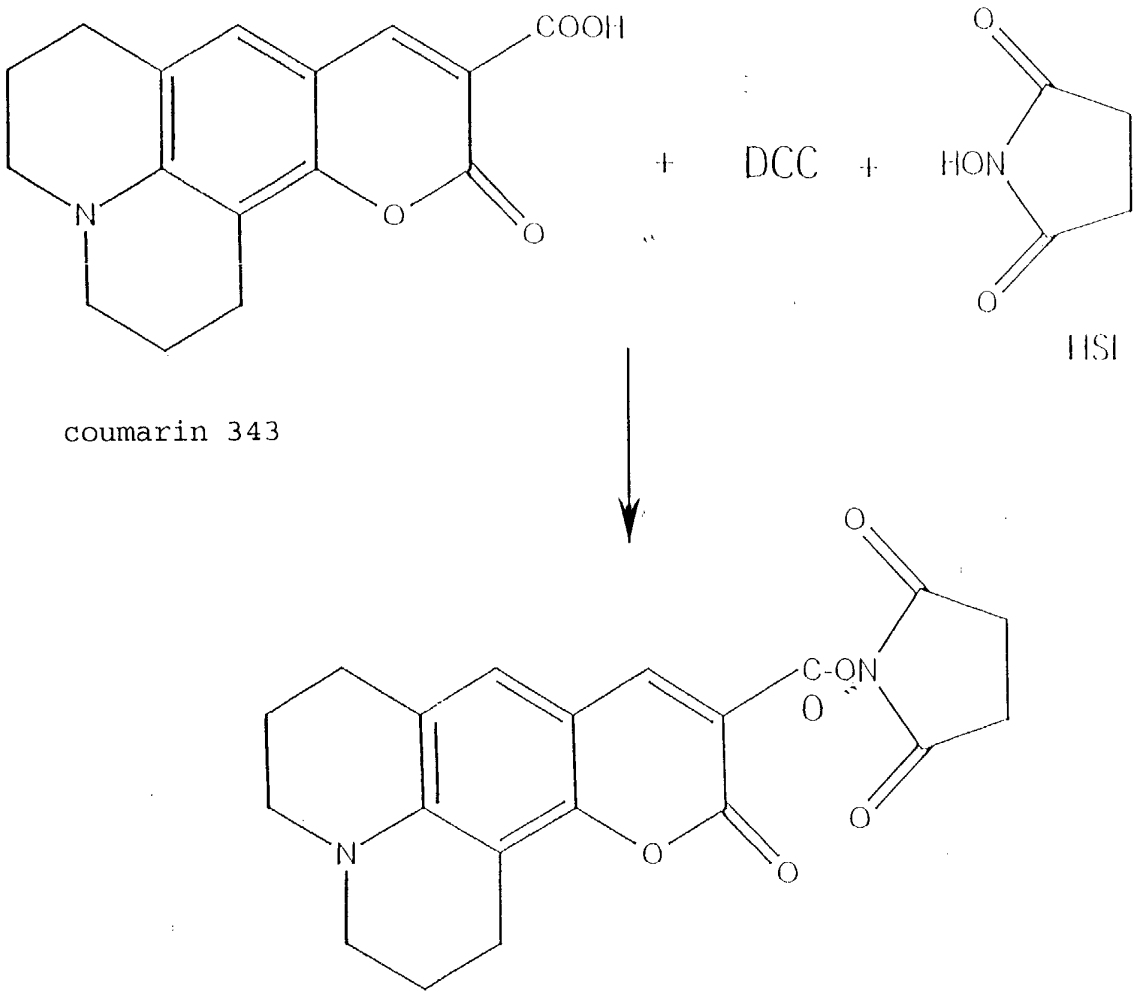
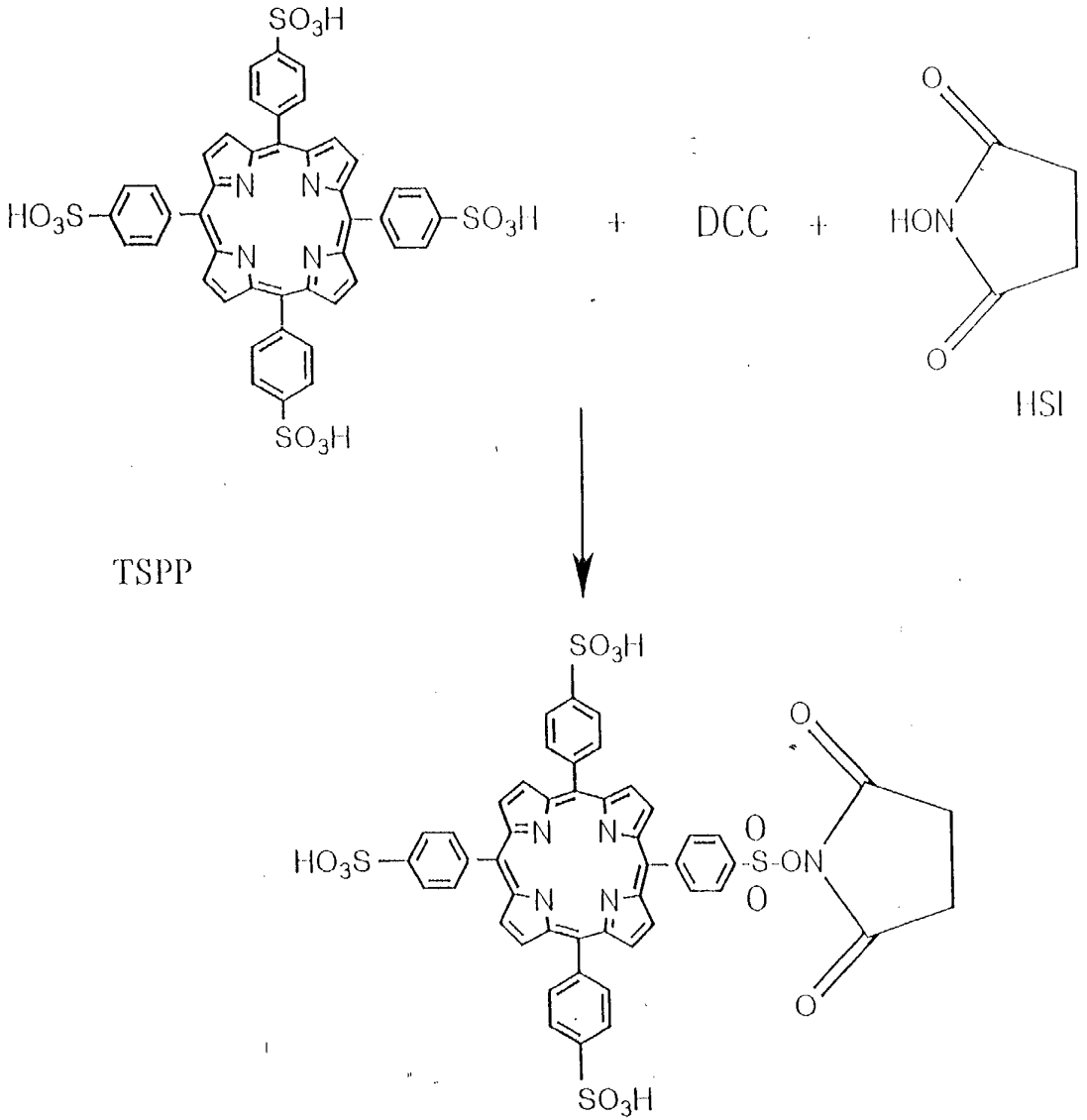


Figure 2 (cont'd)

Figure 3



09/463474

Figure 3 (cont'd)

K 2785 - P 250

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below at 201 et seq. underneath my name.

I believe I am the original, first and sole inventor if only one name is listed at 201 below, or an original, first and joint inventor if plural names are listed at 201 et seq. below, of the subject matter which is claimed and for which a patent is sought on the invention entitled

CONJUGATE FOR DIFFERENTIATING BETWEEN HEALTHY AND UNHEALTHY TISSUE

and for which a patent application:
☐ is attached hereto and includes amendment(s) filed on *(if applicable)*
☒ was filed in the United States on January 21, 2000 as Application No. 09/463,474 *(for declaration not accompanying application)*
with amendment(s) filed on *(if applicable)*
☒ was filed as PCT international Application No. PCT/DE98/02102 on 22 July 1998 and was amended under PCT Article 19 on 9 August 1999 *(if applicable)*

I hereby state that I have reviewed and understand the contents of the above identified application, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED PRIOR TO THE FILING DATE OF THE APPLICATION			
APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
197 31 741.3	Germany	23 July 1997	YES ☒ NO ☐
			YES ☐ NO ☐

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER	FILING DATE

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS		
		PATENTED	PENDING	ABANDONED

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OIPE JC103 AUG 04 2000 PATENT & TRADEMARK OFFICE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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